

REMARKS

Claims 1- 41 are pending in the application. Claims 1- 4, 15-19, 21 and 22 are rejected. Claims 23-41 are withdrawn from consideration as being drawn to a non-elected invention. Claims 5-14 and 20 are withdrawn from consideration as being drawn to a non-elected species. Claim 3 has been canceled without prejudice or disclaimer. Claims 1 and 21 have been amended to better define what Applicants believe to be the invention. Accordingly, claims 1, 2, 4, 15-19, 21 and 22 remain under consideration.

Claim 13 has been rejected under 35 U.S.C. 112, first paragraph for failure to comply with the enablement requirement. Applicants respectfully traverse the Examiner's rejection, and have canceled the claim without prejudice or disclaimer in order to place the application in condition for allowance.

Claims 1, 2, 4, 15-17, 19, 21 and 22 have been rejected under 35 U.S.C. §103(a), as being unpatentable over Albert et al (Journal of Experimental Medicine, 1998, Vol. 188, pp. 1359-1368), in view of the abstract of Kirberg et al. (European Journal of Immunology, 1003, Vol. 23, pp. 1963-1967) and Matzinger (Annual review of Immunology, 1994, Vol. 12, pp. 991-1045) and Migita et al. (J. Clin. Investigation, 1995, Vol. 96, pp. 727-732). Furthermore, claims 1, 2, 4, 15-19, 21 and 22 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Albert et al (Journal of Experimental Medicine, 1998, Vol. 188, pp. 1359-1368), in view of the abstract of Kirberg et al. (European Journal of Immunology, 1003, Vol. 23, pp. 1963-1967) and Matzinger (Annual review of Immunology, 1994, Vol. 12, pp. 991-1045) and Migita et al. (J. Clin. Investigation, 1995, Vol. 96, pp. 727-732) as applied to claims 1, 2, 4, 15-17, 19, 21 and 22 above and further in view of Li et al. (Transplantation 1998, Vol. 66, pp. 1387-1388) and Sehgal et al. (Clin. Biochemistry, 1998, Vol. 31, pp. 335-340). Applicants respectfully traverse the Examiner's rejection and assert that the Albert et al. reference, which is dated October 5, 1998, was published after the filing of provisional applications having U.S. serial numbers 60/075,356 filed February 20, 1998; 60/077,095, filed March 6, 1998; and 60/101,749, filed September 24, 1998, to which the present application claims priority. Accordingly, Applicants assert that it would not be obvious to the skilled practitioner to combine the remaining references to read on the claims of the present

invention without the teachings of Albert et al. Moreover, Applicants assert that there would be no suggestion or motivation to combine the remaining references with any reasonable expectation of success without the teachings of Applicants' own work. Withdrawal of the rejection is respectfully requested.

Claim Rejections under 35 U.S.C. §112

Claim 3 has been rejected under 35 U.S.C. 112, first paragraph for failure to comply with the enablement requirement. In particular, the Examiner alleges that claim 3 requires the absence of T cell help by means of excluding the CD+4 T cells from the matured dendritic cells exposed to apoptotic cells. Moreover, the Examiner alleges that the claim requires the introduction of the dendritic cells into a mammal and as such it is unclear how CD+4 T cell help can be excluded from interacting with the matured dendritic cells after infusion into said mammal without the aid of a drug which is administered in vivo. The Examiner alleges that without further guidance from the specification one of skill in the art would not be able to carry out the invention in vivo without undue experimentation. Applicants respectfully traverse the Examiner's rejection, and have canceled the claim without prejudice or disclaimer in order to place the application in condition for allowance.

Claim Rejections under 35 U.S.C. §103(a)

Claims 1, 2, 4, 15-17, 19, 21 and 22 have been rejected under 35 U.S.C. §103(a), as being unpatentable over Albert et al (Journal of Experimental Medicine, 1998, Vol. 188, pp. 1359-1368), in view of the abstract of Kirberg et al. (European Journal of Immunology, 1003, Vol. 23, pp. 1963-1967) and Matzinger (Annual Review of Immunology, 1994, Vol. 12, pp. 991-1045) and Migita et al. (J. Clin. Investigation, 1995, Vol. 96, pp. 727-732). Furthermore, claims 1, 2, 4, 15-19, 21 and 22 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Albert et al (Journal of Experimental Medicine, 1998, Vol. 188, pp. 1359-1368), in view of the abstract of Kirberg et al. (European Journal of Immunology, 1003, Vol. 23, pp. 1963-1967) and Matzinger (Annual review of Immunology, 1994, Vol. 12, pp. 991-1045) and Migita et al. (J. Clin. Investigation, 1995, Vol. 96, pp. 727-732) as applied to claims 1, 2, 4, 15-17, 19, 21 and

22 above and further in view of Li et al. (Transplantation 1998, Vol. 66, pp. 1387-1388) and Sehgal et al. (Clin. Biochemistry, 1998, Vol. 31, pp. 335-340).

The invention as claimed. The claims of the present application are drawn to methods for inducing tolerance in a mammal to an antigen comprising the steps of: isolating peripheral blood mononuclear cells (PBMC) from a whole blood sample from said mammal; isolating dendritic cells from said PBMC; exposing said dendritic cells *ex vivo* to apoptotic cells expressing said antigen in the presence of at least one dendritic cell maturation stimulatory molecule and in the absence of effective CD4⁺ T cell help, wherein said dendritic cells upon exposure to said dendritic cell maturation stimulatory molecule are characterized as having the phenotype CD14⁻ and CD83⁺; and introducing the dendritic cells into said mammal; wherein said dendritic cells induce apoptosis of antigen-specific CD8⁺ T cells in said mammal resulting in tolerance to said antigen. The dependent claims are drawn to particular dendritic cell maturation factors including PGE2, TNF-alpha, lipopolysaccharide, monocyte conditioned medium, CpG-DNA, or any combination thereof. Additional dependent claims are drawn to methods for exclusion of effective CD4⁺ T cell help by including at least one agent that inhibits or eliminates effective CD4⁺ T cell help. The agent which inhibits or eliminates effective CD4⁺ T cell help inhibits signaling consequent to dendritic cell-CD4⁺ T cell engagement. Such an agent is selected from a FKBP antagonist and a TOR antagonist. The FKBP antagonist is tacrolimus. The TOR antagonist is rapamycin. The antigen is a tumor antigen, a viral antigen, a self-antigen or a transplant antigen. The dendritic cells are infused into the mammal after the dendritic cells mature and exhibit the phenotype CD14⁻ and CD83⁺ and the mammal is a human.

The Albert et al. reference as a whole. The Examiner alleges that Albert et al. teach that dendritic cells phagocytose apoptotic cells and cross-present antigens from the apoptotic cells to cytotoxic T lymphocytes. Moreover, Albert et al teach that dendritic cells can acquire antigen from tumors, transplants, infected cells and self tissues for stimulation or tolerization of T cells. The Examiner further alleges that Albert et al. teach the isolation of dendritic cells from peripheral blood and the use of monocyte conditioned medium as

a maturation factor, and further that the mature cells have the phenotype CD14⁻, CD83⁺ and HLA-Drhi. In addition, Albert et al. teach that the maturation factors are LPS, ceramide, CD40L, TNF-alpha and PGE2 in addition to macrophage conditioned medium, and that the co-culture of immature dendritic cells with apoptotic cells in the presence of macrophage conditioned medium, a maturation stimulus for dendritic cells, made apoptotic cells a better target for cross-presentation of antigen.

Albert et al. **do not teach or suggest** the methods of the present invention for inducing tolerance. In particular, Albert et al. **do not teach that the absence of CD4⁺ T cell help, or the inhibition of CD4⁺ T cell help through the use of inhibitors of signaling consequent to dendritic cell-CD4⁺ T cell engagement is a requirement in conjunction with the generation of CD14⁻ and CD83⁺ dendritic cells following addition of maturation factors** using the methods described in the present application for tolerance induction. In addition, Albert et al. do not teach the inhibition of signaling using agents such as those described herein, such as FK506 or rapamycin.

More importantly, the date of publication of the Albert et al reference was October 5, 1998, which postdates the filing dates of the earlier filed applications to which the present application claims priority. In particular, the Examiner's attention is drawn to the Office Action dated February 27, 2003, for which a response was filed on May 27, 2003, which included an executed oath/declaration, as well as an amendment to the specification to include reference to the related applications to which priority was claimed. In particular, as noted in the amendment filed:

"This application is a continuation-in-part of U.S. Serial No. 09/565,958, filed May 5, 2000, and a continuation-in-part of U.S. Serial No. 09/251,896, filed February 19, 1999, which claims priority to U.S. Provisional applications Serial No. 60/075,356, filed February 20, 1998; and Serial No. 60/077,095, filed March 6, 1998; and Serial No. 60/101, 749, filed September 24, 1998, all of which are incorporated herein by reference in their entireties."

Kirberg et al. reference as a whole. The Kirberg et al. reference teaches that CD4⁺ T cell help prevents deletion of CD8⁺ T cells after transient response to antigen.

Kirberg et al. **do not teach or suggest** the methods of the present invention. In particular, Kirberg et al. **do not teach that the presentation of antigen by apoptotic cells to the dendritic cell with a dendritic cell maturation factor in the absence of CD4+ T cell help results in induction of tolerance.** In particular, Kirberg et al. **do not teach** that following exposure of the dendritic cells to a maturation stimulus in the absence of CD4+ T cell help results in **generation of a population of dendritic cells expressing high levels of surface expression of CD83⁺, but which are CD14⁻.** Kirberg et al. also do not teach that introducing the dendritic cells having these markers into a mammal results in induction of apoptosis of antigen-specific CD8+ T cells in the mammal resulting in tolerance to the antigen. In addition, Kirberg et al. **do not contemplate the use of agents that inhibit or eliminate effective CD4+ T cell help, such as FK506 or rapamycin,** which inhibit or eliminate effective CD4+ T cell help by inhibiting signaling consequent to dendritic cell-CD4+ T cell engagement.

Migita et al. reference as a whole. Migita et al. teach that FK506 enhances the apoptotic effect of anti-CD3 antibody. FK506 by itself does not induce apoptosis.

Migita et al. **do not teach or suggest** the methods of the present invention. In particular, Migita et al. **do not teach** that the presentation of antigen by apoptotic cells to the dendritic cell with a dendritic cell maturation factor in the absence of CD4+ T cell help results in induction of tolerance. In particular, Migita et al. **do not teach** that following exposure of the dendritic cells to a maturation stimulus in the absence of CD4+ T cell help results in generation of a population of dendritic cells expressing high levels of surface expression of CD83⁺, but which are CD14⁻. Furthermore, Migita et al. **do not teach** that the agents of the present invention, eg. tacrolimus or rapamycin, which inhibits or eliminates effective CD4+ T cell help, does so by inhibiting signaling **consequent to dendritic cell-CD4+ T cell engagement.**

Matzinger et al. reference as a whole. Matzinger et al. teach that cytotoxic T lymphocytes become unresponsive to antigen if the cytotoxic T lymphocyte encounters antigen first in the absence of CD4+ T cell help.

Matzinger et al. **do not teach or suggest** the methods of the present invention. More particularly, Matzinger et al. **do not teach** that presentation of antigen for which tolerance is desired via apoptotic cells to dendritic cells, in the presence of a dendritic cell maturation factor, but in the absence of T cell help using agents such as tacrolimus or rapamycin, results in **generation of dendritic cells having a phenotype of CD14⁻, and CD83⁺, which when administered to a subject results in apoptosis of antigen specific T cells**. Matzinger et al. **do not teach** that the agents of the present invention, *eg.* tacrolimus or rapamycin, which inhibits or eliminates effective CD4⁺ T cell help, does so by inhibiting signaling **consequent to dendritic cell-CD4⁺ T cell engagement**.

The Li et al. reference as a whole. Li et al. teach that tolerance to an allograft may be induced using **rapamycin as adjunct therapy with a co-stimulation blockade** (anti-CD40 ligand plus CTLA-4Ig). Furthermore, **Li et al. teach that rapamycin blocks IL-2 induced proliferation, but not apoptotic signals to achieve tolerance to an antigen**.

Li et al. **do not teach or suggest** the methods of the present invention. In particular, Li et al. do not teach the presentation of antigen for which tolerance is desired via apoptotic cells to dendritic cells in the presence of a dendritic cell maturation factor, but in the absence of CD4⁺ T cell help. More particularly, Li et al. do not teach the methods of the present invention which result in the generation of a population of CD14⁻, CD83⁺ dendritic cells, which when administered to a human subject result in apoptosis of antigen specific T cells. Furthermore, Li et al. do not teach that the agents of the present invention, *eg.* tacrolimus or rapamycin, which inhibit or eliminate effective CD4⁺ T cell help, do so by **inhibiting signaling consequent to dendritic cell-CD4⁺ T cell engagement**.

The Sehgal reference as a whole. Sehgal teaches that rapamycin complexes with the immunophilin FKBP to produce the mammalian inhibitor of rapamycin complex which blocks the IL-2 mediated signal transduction pathway that prevents cell cycle progression from G1 to S phase in T cells.

Sehgal et al **do not teach or suggest** the methods of the present invention. In particular, Sehgal does not teach the presentation of antigen for which tolerance is desired

via apoptotic cells to dendritic cells in the presence of a dendritic cell maturation factor, but in the absence of CD4⁺ T cell help. More particularly, Sehgal does not teach the methods of the present invention which result in the generation of a population of CD14⁻, CD83⁺ dendritic cells, which when administered to a human subject result in apoptosis of antigen specific T cells. Furthermore, Sehgal does not teach that the agents of the present invention, eg. tacrolimus or rapamycin, which inhibit or eliminate effective CD4⁺ T cell help, do so by **inhibiting signaling consequent to dendritic cell-CD4⁺ T cell engagement**. In addition, **the target of RAPA:FKBP is distinct from calcineurin**, unlike FK506, which when complexed with its respective immunophilin inhibits calcineurin, which is required for early T cell activation.

The analysis under § 103(a). The references noted herein do not teach or suggest the methods disclosed in the present application for tolerance induction ex vivo. Nor do the references when combined suggest to one skilled in the art how to make or use the invention as currently claimed. Moreover, since the methods described in the present application for tolerance induction were unknown at the time of the references cited, it was not possible to predict the steps and conditions necessary to optimize induction of antigen specific tolerance. Moreover, it was not until Applicants' present invention that the precise steps involved in tolerance induction by presenting antigen to dendritic cells via apoptotic cells in the presence of dendritic cell maturation factors, but in the absence of CD4⁺ T cell help, which then resulted in the generation of dendritic cells bearing the phenotype CD14⁻ and CD83⁺, which upon transfer to a subject resulted in apoptosis of antigen specific CD8⁺ T cells, were identified. Furthermore, it was not until the time of Applicants' own research that it was realized that the absence of CD4⁺ T cell help could be substituted by the use of inhibitors of signaling consequent to dendritic cell-CD4⁺ T cell engagement, such as with tacrolimus or rapamycin. In addition, the present invention teaches, and no prior art suggests, that a specific action of such inhibitors of signaling on a reconstituted system consisting of dendritic cells and apoptotic cells, provides a long-lasting effect on those dendritic cells such that they are now "inhibitory/tolerizing" even with subsequent encounter to CD4.

Furthermore, Applicants assert that the rejection under 35 U.S.C. 103(a) is improper in light of the claim for priority of the instant application to the earlier filed applications having U.S. serial number 09/565,958, filed May 5, 2000 and U.S. serial number 09/251,896, filed Feb. 19, 1999, both of which claim priority to provisional application numbers 60/075,356, filed February 20, 1998; serial number 60/077,095, filed March 6, 1998 and serial number 60/101,749, filed September 24, 1998.

More particularly, the use of apoptotic cells for delivery of antigen to dendritic cells for cross-priming or cross-tolerance was first described in Provisional applications having serial numbers 60/075,356, filed February 20, 1998; 60/077, 095, filed March 6, 1998 and 60/101,749, filed September 24, 1998, to which the present application claims priority. The Examiner's attention is drawn to USSN 60/077,095 page 15, lines 3-8 where this concept of cross priming and cross-tolerance is described. Furthermore, the CD14 and CD83 markers that define the level of maturation in a dendritic cell is described in USSN 60/101,729 on page 71, lines 24-34 and on page 72, lines 0-4 and further on page 74, lines 15-17.

In addition, USSN 09/251,896, which has a filing date of February 19, 1999, describes cross tolerance on page 64, lines 21-32, immature dendritic cell markers on page 75, lines 10-26 and the induction of maturation of the dendritic cells by certain maturation factors is described on page 91, lines 0-28. Accordingly, since the present application was filed within one year of the publication of Applicants' own reference cited herein (Albert et al.), which was October 5, 1998, and since the USSN 09/251,896 application claims priority to the earlier filed provisional applications, as noted above, Applicants assert that the inclusion of this reference in the 35 U.S.C. 103(a) rejection is improper. Withdrawal of the rejection is respectfully requested.

Thus, the connection between the work done by Albert et al (Journal of Experimental Medicine, 1998, Vol. 188, pp. 1359-1368), in view of the abstract of Kirberg et al. (European Journal of Immunology, 1993, Vol. 23, pp. 1963-1967) and Matzinger (Annual Review of Immunology, 1994, Vol. 12, pp. 991-1045) and Migita et al. (J. Clin. Investigation, 1995, Vol. 96, pp. 727-732) as applied to claims 1, 2, 4, 15-17, 19, 21 and 22 is moot, given the fact that the Albert et al reference was published after the earliest effective filing date of the present application.

Likewise, the same would hold true for the rejection of claims 1, 2, 4, 15-19, 21 and 22 whereby the Examiner alleges that these claims are not patentable over Albert et al. in view of Kirberg et al and Matzinger et al. and Migita et al as applied to claims 1, 2, 4 15-17, 21 and 22, and further in view of Li et al. and Sehgal et al.

As noted by the Examiner, the test for obviousness is what the combined teachings of the references would have suggested to those of ordinary skill in the art. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981). Applicants assert that without the teachings of Albert et al, it would not be obvious to one skilled in the art to combine the teachings of Kirberg et al., Matzinger et al., and Migita et al., and further in view of Li et al. and Sehgal et al. to result in the teachings of the present application. There would be no suggestion or motivation to combine any of these references with any reasonable expectation of success, which would result in the methods of the present invention, as currently claimed.

Applicants contend that given the fact that the Albert et al. reference postdates the earlier filed applications, to which the present application claims priority, the remaining references, when combined, would not motivate or suggest to a person skilled in the art how to teach or practice the methods of the instant invention, as currently claimed. It was only from Applicants' own teachings that the claimed invention could be appreciated and practiced.

In light of the foregoing claim amendments and arguments, Applicants respectfully request withdrawal of the rejection.

Fees

No fees are believed to be necessitated by the instant response. However, should this be in error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment, or to credit any overpayments.

Conclusion

Applicants believe that in view of the foregoing, the claims are in condition for allowance. Withdrawal of the rejections is respectfully requested. If a discussion with the undersigned will be of assistance in resolving any remaining issues, the Examiner is invited to telephone the undersigned at (201) 487-5800, ext. 118, to effect a resolution.

Respectfully submitted,



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